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14. ABSTRACT
Androgen receptor (AR) plays a central role in prostate cancer and the regulation of its nuclear/cytoplasmic transport represents another level control of its activity. However, how AR is transported into and out of the nucleus remains largely a mystery. Yeast (S. cerevisiae) provides a powerful system to study fundamental cellular mechanisms. Thus, we use yeast as a model system to identify factors that are involved in the AR nuclear export. A mutagenesis screen was performed and thus far, 6000 individual clones (about 1 yeast genome) have been examined. Seven mutants that displayed a defect in nuclear export were isolated. Preliminary results suggest that one mutant yeast clone, 27.7D, is a recessive gene that may play a role in the nuclear localization of AR. In addition, the mutant clone 27.7D also exhibit retarded growth. The export and growth defects observed in clone 27.7D appear to be caused by the same mutation or two separate, but very closely linked mutations. Complementation experiments show that 27.7D is distinct from two other mutants, clones 1.3E and 72.5E, which are actively being characterized. Initial results also suggest that the combination of the mutation in 27.7D and the mutation of another mutant clone 102.5D results in lethality. This suggests the possibility that these two genes may belong to two parallel pathways.

15. SUBJECT TERMS
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Introduction

In the United States, prostate cancer is the most common nonskin cancer and the second leading cause of cancer death in males. Although it is well established that androgen receptor (AR) plays a central role in prostate cancer and that its subcellular localization is important for the regulation of its activity [1], relatively little is known about AR intracellular trafficking. Since yeast provides a powerful system to study fundamental cellular mechanisms, we decided to use yeast genetics to identify factors that are involved in the AR nuclear export.

Body

Previous work in our lab demonstrated that a novel nuclear export signal for AR (NES^{AR}) was both necessary and sufficient for AR nuclear export in mammalian cells [2]. Also, our preliminary experiments showed that this sequence is capable of directing cytoplasmic localization of a GFP (Green Fluorescent Protein) tag and is also functionally dominant over the nuclear localization sequence from SV40, NLS^{SV40}, in yeast.

To identify molecular factors that are involved in AR nuclear export, a yeast genetic screen was performed first to look for mutants that fail to export the NES^{AR}. According to our proposal, wild-type yeast was transformed with p416 GPD-GFP-NLS^{SV40}-GFP-NES^{AR}. In wild-type yeast, this fusion protein transformed yeast strain was then mutagenized using EMS (Ethyl Methane-sulfonate) [3]. Thus far, 6000 individual clones (about 1 yeast genome) have been screened under fluorescence microscopy and 7 candidates that failed to display cytoplasmic GFP localization have been isolated. According to my **Statement of Work**, I mainly focused on two yeast mutants, 27.7D and 102.5D. Both of them exhibit nuclear localization of the GFP-NLS^{SV40}-GFP-NES^{AR} fusion protein. Retransformation with a fresh fusion protein construct (not exposed to mutagen), p416 GPD-GFP-NLS^{SV40}-GFP-NES^{AR}, confirmed that the mutations of 27.7D and 102.5D are genomic mutations rather than mutations in the plasmid (Fig. 1).

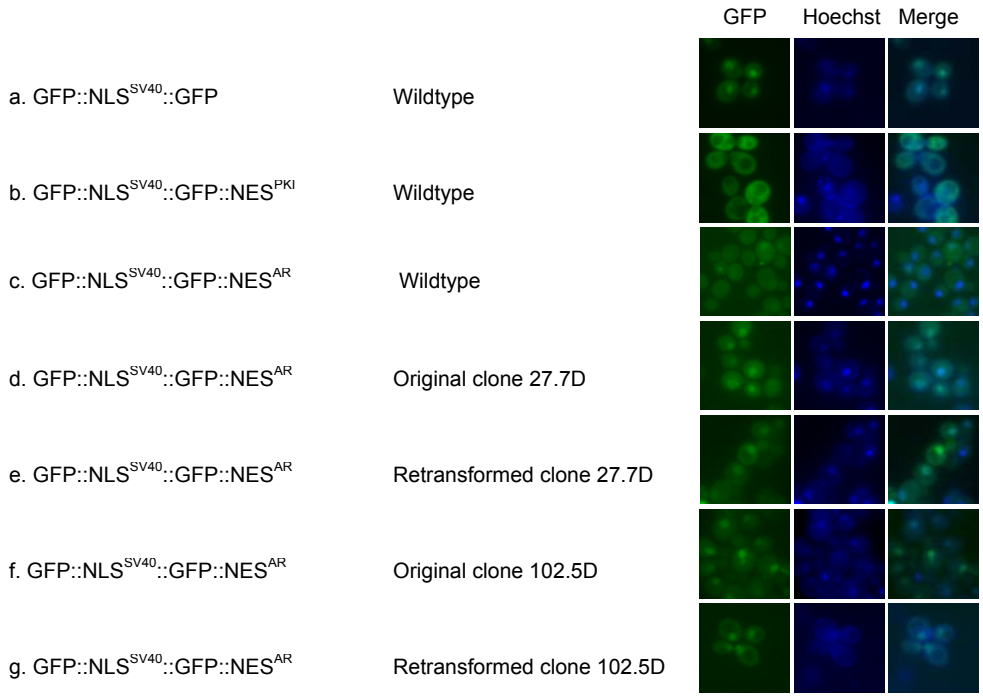

Fig. 1. GFP localization of test constructs, mutant yeast clones 27.7D and 102.5D. Yeast strain BY4741 was transformed with the indicated constructs and nuclei were stained with Hoechst. GFP localization and Hoechst staining were viewed under fluorescence microscopy. a) Wild-type yeast transformed with NLS^{SV40} alone showed nuclear localization. b) Wild-type yeast transformed with NLS^{SV40} and NES^{PKI} and c) Wild-type yeast transformed with NLS^{SV40} and NES^{AR} abrogated NLS^{SV40} nuclear localization. d) Original clone 27.7D containing NLS^{SV40} and NES^{AR} displayed nuclear GFP localization. e) Original clone 27.7D that lost NLS^{SV40} and NES^{AR} was retransformed with NLS^{SV40} and NES^{AR}, the retransformed clone 27.7D still showed nuclear GFP localization. f) Original clone 102.5D containing NLS^{SV40} and NES^{AR} displayed nuclear GFP localization. g) Original clone 102.5 that lost NLS^{SV40} and NES^{AR} was retransformed with NLS^{SV40} and NES^{AR}, the retransformed clone 102.5D still showed nuclear GFP localization.

Complementation tests (meiotic analyses) were performed to determine if the mutant phenotype (nuclear localization of GFP) is caused by an alteration at a single genetic locus. Meiotic analysis here was carried out by backcrossing mutant strain to a wild type strain. The diploid cross was isolated and sporulated. The resulting haploid spores are allowed to germinate and form individual colonies on complete medium. Then the haploid spores can be scored for the phenotypes. Because the four spores from each tetrad are the product of a single meiotic event, a 2:2 segregation of mutant to wild-type phenotypes is indicative of a single gene [4]. Currently I have already completed the 3rd backcross of mutant 27.7D (N=40) (Fig. 2). My preliminary results have indicated that GFP localization in mutant yeast 27.7D does display typical 2:2 segregation after the 3rd backcross. It also has been found that mutant 27.7D results in retarded growth of yeast, and the GFP localization is closely linked to the spore colony size although the difference among colony sizes is relatively subtle (Fig. 3). Since there were still a few tetrads (2/40) whose GFP localization does not show distinct 2:2 segregation. Neither does their colony size. I decided to do the 4th backcross, and try to get rid of some potential background mutations.

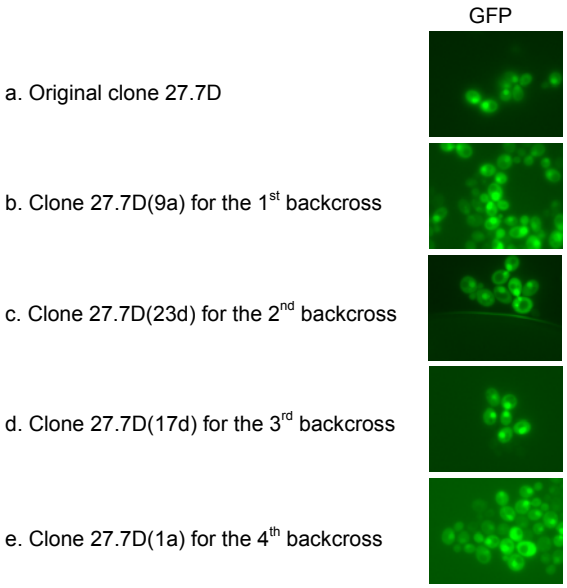

Fig. 2. GFP localization of mutant yeast clones 27.7D. GFP localization was viewed under fluorescence microscopy. a) The original mutant clone 27.7D retransformed with p416 GPD-GFP-NLS^{SV40}-GFP-NES^{AR}. b) After crossing the original clone 27.7D to wild-type yeast strain, clone 27.7D(9a) was chosen for the 1st backcross. c) After crossing the clone 27.7D(9a) to wild-type yeast, clone 27.7D(23d) was selected for the 2nd backcross. Similarly, d) 27.7D(17d) was derived from 27.7D(23d), and 27.7D(1a) was from 27.7D(17d) for next backcross.

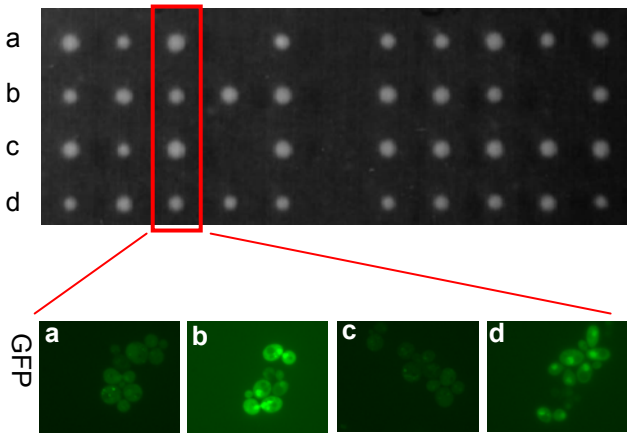

Fig. 3. Representation tetrad: 27.7D X Wild type. The mutant clone 27.7D was crossed with wild type strain BY4741 to generate diploid cross 27.7D X BY4741, which was dissected to produce haploid progenies. We named the four spores of a tetrad as a, b, c, d, respectively. Top panel: the spore colonies derived from asci separated on the surface of a petri dish. Bottom panel: GFP localization of each haploid spore containing p416 GPD-GFP-NLS^{SV40}-GFP-NES^{AR} viewed under fluorescence microscopy.

I also performed a similar meiotic analysis with the mutant clone 102.5D. As illustrated in Fig. 4, the colony size of haploid spores derived from the cross of 102.5D X wild-type also shows typical 2:2 segregation of small to large. The GFP localization phenotype of 27.7D was more distinctive than that of 102.5D, therefore I chose concentrate my efforts in identifying this mutation. However, I am continuing to characterize mutant 102.5D in parallel.

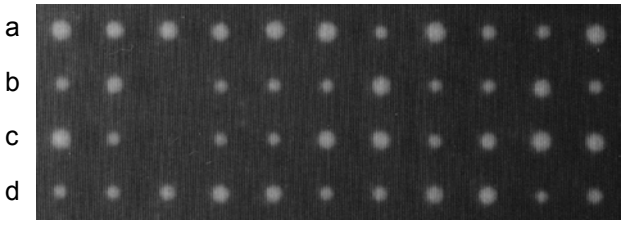

Fig. 4. Representation tetrad: 102.5D X Wild type. The spore colonies derived from asci separated on the surface of a petri dish. The mutant clone 102.5D was crossed with wild type strain BY4742 to generate diploid cross 102.5D X BY4742, which was dissected to produce haploid progenies. We named the four spores derived from a tetrad as a, b, c and d, respectively.

To address which gene has been mutated, we will first need to determine the dominant/recessive character of the mutations, and place different mutants into complementation groups. The dominant or recessive character is revealed by crossing the mutant strain with wild type strain to form a diploid cell. A mutation is dominant when the mutant phenotype is expressed in a heterozygous diploid cell. A mutation is recessive when the diploid has the same phenotype as the wild type strain [5]. Then the pair-wise complementation tests between all the mutant strains (and also with wild type) were carried out. The results are illustrated in Table 1 and Fig. 5, where "C" indicates the wild type phenotype (Cytoplasmic GFP localization) and "N" indicates the mutant phenotype (Nuclear GFP localization). The mutant yeast clones 72.5E and 1.3E are two other potential candidates that are being actively investigated by Dr. Minh Nguyen. The reason that I have crossed the clone 27.7D, 102.5D with those two mutant clones is we would like to know if these mutants are resulted from mutations in the same or different genes. My findings showed that clones 27.7D and 72.5E contain recessive mutations. 1.3E bears a dominant mutation. The complementation group of clone 27.7D is different from either clone 72.5E or 1.3E. Usually one complementation group is equivalent to one gene.

	Wild type	27.7D	102.5D	72.5E	1.3E
Wild type	C	C	Ongoing	C	N
27.7D	C	N	C	C	Ongoing
102.5D	Ongoing	C	Ongoing	C	N
72.5E	C	C	C	–	–
1.3E	N	Ongoing	N	–	N

Table 1. Summary of complementation tests.

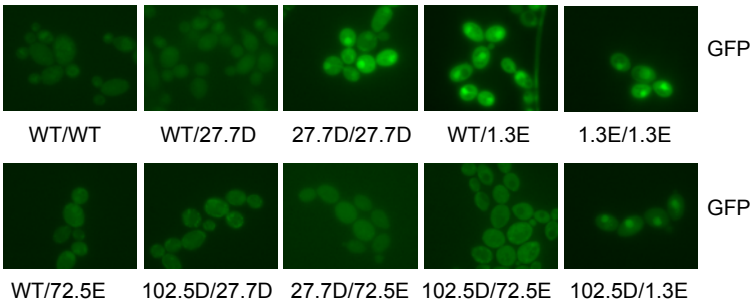

Fig. 5. GFP localization of diploid crosses. WT: wild type strain; /: cross.

To investigate the chromosomal locations of mutated gene of clone 27.7D and 72.5E, the diploid cross of 27.7D X 72.5E was sporulated, the phenotype of sixty two tetrads were scored under fluorescence microscopy. My findings showed that the number of tetrads showing Parental Ditype (PD) is 16, the number of Nonparental Ditype (NPD) is 14, and the number of tetratype is 32. Thus, the ratio PD: NPD: T is approximately 1:1: <4 which suggested that those two genes are on

separate chromosomes and both genes are linked to their centromeres [4] [6]. Regarding the results of cross of 27.7D X 1.3E, Fifty six tetrads were analyzed. The number of PD is 16, the number of NPD is 4, and the number of T is 36. The ratio of PD: NPD: T is approximately 1:< 1, we can conclude that those two genes are close to each other (that is, “linked”) on the same chromosome [4] [6]. After calculation, the map distance between those two genes is about 60 cM (centimorgans).The equation for deducing map distances is: cM = 100/2 [(T+6NPD) / (PD+NPD+T)]. For larger distances up to approximately 75cM, the value can be corrected by the equation: cM (corrected) = [(80.7)(cM)-(0.883)(cM)^2] / (83.3-cM) [4].

The preliminary results about the cross of 27.7D X 102.5D showed that 27.7D and 102.5D double mutation probably results in lethality (Fig. 6). This might suggest that the gene product of mutant 27.7D operates in a pathway parallel to the one that is affected by mutant 102.5D. But the further investigations still need to be done.

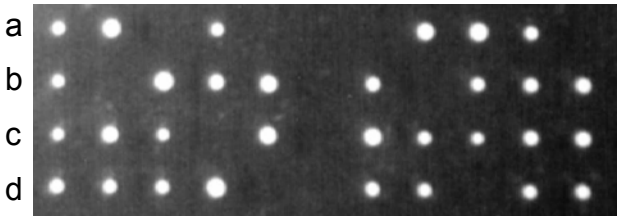

Fig. 6. Representation tetrad: 27.7D X 102.5D. The spore colonies derived from asci separated on the surface of a petri dish. The mutant clone 27.7D was crossed with mutant clone 102.5D to generate diploids, which was dissected to form haploid progenies.

Key Research Accomplishments

The mutant yeast clone 27.7D represents a recessive gene controlling the nuclear localization of AR. The mutant clone 27.7D results in retarded growth of yeast. Export defect and growth retardation observed in clone 27.7D appear to be a result of the same mutation or two different, but very closely linked mutations.

The mutated genes of 27.7D and 72.5E are located on different chromosomes and both genes are linked to their centromeres.

The mutant genes of 27.7D and 1.3E are close to each other on the same chromosome, the map distance between these two genes is approximately 60cM.

Reportable Outcomes

Mutant yeast strains defective for NES^{AR} mediated nuclear export have been isolated.

Conclusion

Currently, the mechanisms by which AR is exported from the nucleus remain largely unknown. In this proposal we presented evidence for the feasibility of using *S. cerevisiae* as a genetic tool to identify and characterize proteins that are required for the nuclear export mediated by NES^{AR}. Understanding the AR export pathway may lead to additional targets for the treatment of prostate cancer as well as other diseases caused by the deregulation of steroid receptors. According to our current experiment progress, we should be able to complete our project on time.

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Wang, Yujuan	Post-doctoral fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

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Lanzhou Medical College, Gansu Province, China	MS	1996-1999	Dermatology and Venerology
Institute of Dermatology, Peking Union Medical College, Jiangsu Province, China	PhD	1999-2003	Dermatology and Venerology
Northwestern University, Feinberg School of Medicine, Chicagao, IL	Post-doc	2004-2006	Urology
University of Pittsburgh school of Medicine, Pittsburgh, PA	Post-doc	2006-Present	Urology

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Positions and Employment

1996 — 1999 Graduate Research, Teaching Assistant and Resident/Dermatology and Venerology, Lanzhou Medical College, Gansu Province, China

1999 — 2003 Graduate Research, Teaching Assistant and Dermatologist, Institute of Dermatology, Peking Union Medical College, Jiangsu Province, China

2004 — 2006 Post-doctoral Fellow, Department of Urology, Northwestern University, Chicago, IL

2006 — Post-doctoral Fellow, Department of Urology, University of Pittsburgh, Pittsburgh, PA

Honors

2005 — Recipient of a Post-doctoral Fellowship from DOD

B. Selected peer-reviewed publications (in chronological order).

1. ***Yujuan Wang***, Jiarun Zheng. The progression of thalidomide in recent 3 years, Foreign Medical Sciences (Dermatology and Venerology), 26: 329~332, 2001
2. Junkui Ai, Xiao Huang, ***Yujuan Wang***, et al. Screening of novel genes differentially expressed in human renal cell carcinoma by suppression subtractive hybridization, Chinese Journal of Cancer, 21: 1065~1069, 2002

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4. ***Yujuan Wang***, Sanmao Wu, Zhicheng Luo. The levels and clinical significance of IL-2, IFN-γ, IL-12 and TNF-α in peripheral blood of patients with psoriasis vulgaris, China Journal of Leprosy and Skin Diseases, 18: 327~329, 2002
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